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Chemical constituents from the fruit of *Zizyphus jujuba* Mill. var. *spinosa*

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ABSTRACT

Twenty-one compounds, including ten triterpenoids (**1**–**10**), two sterols (**11** and **12**), six flavonoids (**13**–**18**), and three cerebrosides (**19**–**21**) were isolated from the fruit of *Zizyphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex. H. F. Chou. The structures were elucidated by spectroscopic methods and by comparison of their reported spectral data. These compounds have shown the relationship between this plant and other species from the Rhamnaceae family.

1. Subject and source

Zizyphus genus (Rhamnaceae) includes about 170 species, some of which are important economic plants. Among them, 18 species grow in China, 14 of which are indigenous (Ji et al., 2012). *Zizyphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex. H. F. Chou is a small tree or spiny bush widely distributed in northern China (Wu et al., 2013). Its dried seeds (Semen Ziziphi Spinosae), which are called suanzaoren in Chinese, have been used as traditional sedative medicine to treat anxiety, nervousness and sleep-related problems for more than one thousand years (Sun et al., 2011), while its dried fruit (Fructus Ziziphi Spinosae) is recorded as a folk medicine for hemorrhage and diarrhea (Zhonghua Bencao, 1999).

The fruit of *Z. jujuba* Mill. var. *spinosa* was purchased in Xingtai, Hebei Province of China in November, 2012 and was identified by Prof. Deyun Wang, College of Veterinary Medicine, Nanjing Agricultural University. A voucher specimen was deposited in the Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University (specimen No. ZJ-20121102).

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2. Previous work

Previous chemical investigations on genus *Zizyphus* indicated the presence of triterpenoid acids (Yagi et al., 1978; Su et al., 2002), triterpenoid saponins (Maciuk et al., 2004; Yoshikawa et al., 1991), cyclopeptide alkaloids (Suksamrarn et al., 2005; Han et al., 2011), as well as flavonoids (Meng et al., 2013; Pawlowska et al., 2009). Up to now, more than fifty constituents have been isolated from *Z. jujuba* Mill. var. *spinosa*, which can be classified into triterpenoid saponins (Matsuda et al., 1999; Yoshikawa et al., 1997), flavonoids (Cheng et al., 2000; Xie et al., 2011), triterpenoid acids (Guo et al., 2011), alkaloids (Xie et al., 2011; Han et al., 1990) and sterols (J.R. Wang et al., 2008). However, most of the studies were focus on the seeds, while the others had reported the compounds from other parts of this plant, such as fruit and roots.

3. Present study

The hardcore-removed and crashed fruit (20 kg) were extracted twice by 95% aqueous ethanol (v/v 60 L) and by 85% aqueous ethanol (v/v 60 L) for one time. The combined extraction was then concentrated under reduced pressure to give a residue (2.5 kg), which was further partitioned with petroleum ether (PE, boiling point 60–90 °C), ethyl acetate (EtOAc) and *n*-BuOH, successively. The PE portion (380 g) was chromatographed on a silica column, with PE-EtOAc (10:0, 8:2, 7:3, 5:5, 3:7 and 0:10) as eluant to yield six fractions (Fractions 1-6) by TLC analysis. Fraction 2 was then subjected to silica gel column, using PE-EtOAc (from 5:1 to 0:1) as eluant. Compound **1** (18 mg) and **2** (310 mg) were obtained from sub-fraction 2-1 and were purified by recrystallization from the PE-EtOAc mixed solvent. Fraction 3 was chromatographed on a silica gel column and eluted with PE-Acetone (from 10:1 to 1:1) to obtain three groups (from Group 3-1 to Group 3-3). Group 3-2 was further separated on silica gel column by PE-Acetone (from 5:1 to 0:1) and recrystallized respectively from EtOAc, methanol (MeOH) and PE-EtOAc to obtain compound **3** (32 mg), **4** (89 mg) and **6** (12 mg). Fraction 4 was fractioned by silica gel column chromatograph (CC) and eluted with dichloromethane-methanol (CH_2Cl_2–MeOH, from 100:0 to 10:1) to give six sub-fractions (from Sub-fraction 4-1 to Sub-fraction 4-6). Compound **5** (112 mg), **9** (20 mg) and **10** (17 mg) were obtained from 4-3 on silica gel CC with CH_2Cl_2–MeOH (from 50:1 to 5:1) and recrystallization with MeOH respectively. Fraction 6 was grouped into five sub-fractions (from Sub-fraction 5-1 to Sub-fraction 5-5) using CH_2Cl_2–MeOH (from 25:1 to 5:1). Sub-fraction 5-4 was retreated by prepared TLC and purified on Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) as eluant to obtain compound **7** (174 mg) and **8** (16 mg). The EtOAc portion (240 g) was chromatographed on a silica column, with PE-EtOAc (10:0, 7:3, 5:5, 3:7 and 0:10) as eluant to yield five fractions (Fractions A to E) by TLC analysis. Fraction B was subjected on silica gel column and isolated into five groups (from Sub-fraction B-1 to Sub-fraction B-5) with CH_2Cl_2–MeOH (from 15:1 to 1:1). Sub-fraction B-5 was further separated by prepared silica gel TLC and purified on Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) as eluant to yield compounds **11** (21 mg), **12** (14 mg) and **13** (34 mg). Fraction C was subjected on silica gel column and isolated into four groups (from Sub-fraction C-1 to Sub-fraction C-4) with CH_2Cl_2–MeOH (from 15:1 to 0:1). Sub-fration C-1 was further isolated by prepared TLC with gradient PE-EtOAc-MeOH (10:1:0.1 to 1:1:0.1) to give four sub-fractions (from Sub-fractions C-1a to C-1d). Sub-fractions C-1a and C-1c were further performed on Sephadex LH-20 column with CH_2Cl_2–MeOH and recrystallized from MeOH to yield **14** (14 mg) and **15** (21 mg). Sub-fraction C-2 was further purified by Sephadex LH-20 column eluting with CH_2Cl_2–MeOH as eluant and recrystallized by MeOH to give **16** (25 mg), **17** (13 mg) and **18** (19 mg), respectively. Fraction E was fractionated by a silica column eluted by CH_2Cl_2–MeOH (from 8:1 to 1:1) to yield compounds **19** (13 mg), **20** (20 mg) and **21** (16 mg).

The isolated compounds were identified as ceanothenic acid (**1**), ursolic acid (**2**), betulin (**3**), betulinic acid (**4**), alphitolic acid (**5**), alphitolic acid methyl ester (**6**), oleanolic acid (**7**), epiceanothic acid (**8**), ceanothic acid (**9**), platanic acid (**10**), stigmast-5-en-3β, 7α-diol (**11**), stigmast-5, 22-ene-3β, 7α-diol (**12**), 5, 7, 3′, 4′-tetramethoxycatechin (**13**), pinocembrin (**14**), 7, 4′-dihydroxy-5-methoxy flavanone (**15**), (+)-catechin (**16**), (−)-epiafzelechin (**17**), (+)-afzelechin (**18**), 1-O-β-D-glucopyranosyl-(2S, 3R, 4E, 8Z)-2-[2′(R)-hydroxyhexadecanoyl-amino]-4, 8-octadecadiene-1, 3-diol (**19**), 1-O-β-D-glucopyranosyl-(2S, 3R, 4E, 8Z)-2- [2′(R)-hydroxyhoctadecanoyl-amino]-4, 8-octadecadiene-1, 3-diol (**20**), 1-O-β-D- glucopyranosyl-(2S, 3S, 4R, 8Z)-2-[2′(R)-hydroxylignoceno-amino]-8-octadecene-1, 3, 4-triol (**21**), respectively, on the basis of their ^{1}H NMR, ^{13}C NMR, MS, and IR spectra analysis and by comparison with those reported data in the related literature (Fig. 1).

4. Chemotaxonomic significance

The present study reports the isolation and structure elucidation of twenty-one secondary metabolites from the fruit of *Z. jujuba* Mill. var. *spinosa*, including ten triterpenoids (**1**–**10**), two sterols (**11** and **12**), six flavonoids (**13**–**18**), and three cerebrosides (**19**–**21**). This is the first report of compounds **19**–**21** from the Rhamnaceae family, the first report of compounds **11**–**15** from *Zizyphus* and the first report of compounds **1** and **10** from *Z. jujuba* Mill. var. *spinosa*.

Compounds **1**–**10** are different types of pentacyclic triterpenoids: compounds **1**, **8** and **9** belong to the ceanothane type, compounds **3**–**6** and **10** have a lupane skeleton, compound **2** belongs to the ursane group, while compound **7** has an oleanane structure. Compounds **2**–**9** have been reported from *Z. jujuba* Mill. (Guo, 2009; Lee et al., 2004) and *Z. jujuba* Mill. var. *spinosa* (Guo et al., 2011; Li et al., 2005). Compounds **1** and **9** have been isolated from *Zizyphus mauritiana* Lam (Ji et al., 2012), *Zizyphus cambodiana* Pierre (Suksamrarn et al., 2006) and *Alphitonia philippinensis* Braid (Jou et al., 2004), which all belong to Rhamnaceae. Compounds **2**–**6** were reported from other species of Rhamnaceae including *Zizyphus joazeiro* Mart. (Schühly et al., 1999; Leal et al., 2010), *Z. mauritiana* Lam (Ji et al., 2012) and *Z. cambodiana* Pierre (Arai et al., 2008; Suksamrarn et al.,

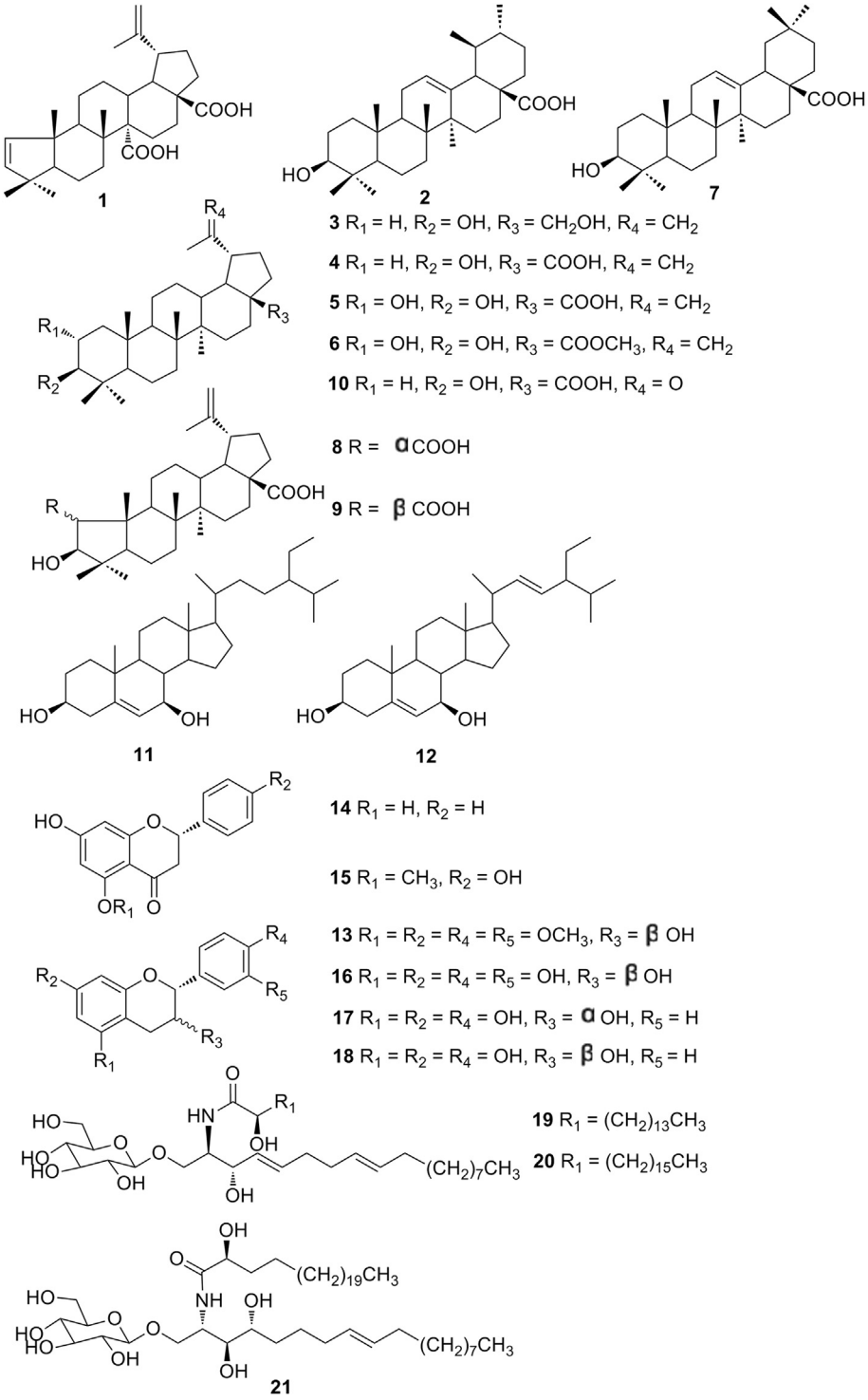

Fig. 1. Structures of compounds **1**–**21**.

2006), *Ampelozizyphus amazonicus* Ducke (Rhamnaceae) (Rosas et al., 2007), as well as *Licania heteromorpha* Bentham var. *heteromorpha* (Chrysoblanaceae) (Braca et al., 2000). Compound **7** was previously isolated from *Psidium guajava* Linn. (Myrtaceae) (Begum et al., 2007). Compound **10** was also obtained from *A. philippinensis* Braid (Jou et al., 2004). It was suggested that the ceanothane-type triterpenoids were the biomarker of the Rhamnaceae family (Guo et al., 2011) and the

data presented here would support this conclusion. Moreover, some other lupane type derivatives had been obtained from other species of *Zizyphus*, for example *Z. joazeiro* Mart. (Schühly et al., 1999), *Z. jujuba* Mill. var. *spinosa* (Lee et al., 1996) and *Zizyphus spina-christi* (L.) Willd (Weinges and Schick, 1995). Therefore, it may be suggested that lupane type triterpenes can serve as chemotaxonomic markers for species of *Zizyphus*.

Compounds **11** and **12** are sterols reported from this genus for the first time. Compound **11** was isolated from *Sedum lineare* Thunb. (Crassulaceae) (Niu et al., 2011), while compound **12** was obtained from *Abelmoschus esculentus* (L.) Moench (Malvaceae) (Jia et al., 2010). Therefore these compounds have a wider distribution to other plant families.

The flavonoid compounds **13**–**18** can be divided into flavanones (**14** and **15**) and flavan-3-ols (**13**, **16**–**18**). Compound **14** was isolated from *Carya cathayensis* Sarg. (Juglandaceae) (Tian et al., 2014) and *Penthorum chinense* Pursh (Saxifragaceae) (Wang et al., 2014), while compound **15** was obtained from *Alpinia katsumadai* Hayata (Zingiberaceae) (X.Q. Wang et al., 2008). Compound **13** was isolated from *Illicium micranthum* Dunn (Illiciaceae) (Geng, 2009), and compounds **16**–**18** were reported from *Z. jujuba* Mill., *Z. jujuba* Mill. var. *spinosa* and *Z. jujuba* Mill. var. *inermis* (Meng et al., 2013). Further research is needed to evaluate the importance of flavan-3-ols as chemical markers in the genus *Zizyphus*.

The cerebroside compounds **19**–**21** were firstly reported from Rhamnaceae family. To date, compounds **19** and **20** have been reported in *Homalomena gigantean* Engl. (Araceae) (Wu et al., 2008). Compound **21** was isolated from the leaves of *Helicia nilagirica* Beed (Proteaceae) (Wu et al., 2004). Two cerebrosides have been obtained from *Z. jujuba* Mill. (Guo, 2009). Therefore, the presence of cerebrosides in both *Z. jujuba* Mill. var. *spinosa* and *Z. jujuba* Mill. could indicate a close phylogenetic relationship between the two species.

This study extends the knowledge about the constituents of *Z. jujuba* Mill. var. *spinosa*. It suggests that lupane type compounds, flavan-3-ols and cerebrosides might have a role as chemotaxonomic markers for *Zizyphus*.

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References

Arai, M.A., Tateno, C., Hosoya, T., Koyano, T., Kowithayakorn, T., Ishibashi, M., 2008. Bioorg. Med. Chem. 16, 9420.
Begum, S., Ali, S.N., Hassan, S., Siddiqui, B.S., 2007. Nat. Prod. Res. 21, 742.
Braca, A., Morelli, I., Mendez, J., Battinelli, L., Braghiroli, L., Mazzanti, G., 2000. Planta Med. 66, 768.
Cheng, G., Bai, Y.J., Zhao, Y.Y., Tao, J., Liu, Y., Tu, G.Z., Ma, L.B., Liao, N., Xu, X.J., 2000. Tetrahedron 56, 8915.
Geng D., 2009. China Pharmaceutical University, 15.
Guo S., 2009. Nanjing University of Traditional Chinese Medicine, 20.
Guo, S., Duan, J.A., Tang, Y.P., Qian, Y.F., Zhao, J.L., Qian, D.W., 2011. Biochem. Syst. Ecol. 39, 880.
Han, B.H., Park, M.H., Han, Y.N., 1990. Phytochemistry 29, 3315.
Han, J., Ji, C.J., He, W.J., Shen, Y., Leng, Y., Xu, W.Y., Fan, J.T., Zeng, G.Z., Kong, L.D., Tan, N.H., 2011. J. Nat. Prod. 74, 2571.
Jia, L., Li, D., Jing, L.L., Guo, M.M., 2010. Zhongyaocai 33, 1262.
Ji, C.J., Zeng, G.Z., Han, J., He, W.J., Zhang, Y.M., Tan, N.H., 2012. Bioorg. Med. Chem. Lett. 22, 6377.
Jou, S.J., Chen, C.H., Guh, J.H., Lee, C.N., Lee, S.S., 2004. J. Chin. Chem. Soc. Tai. 51, 827.
Leal, I.C., dos Santos, K.R., Júnior, I.I., Antunes, O.A., Porzel, A., Wessjohann, L., Kuster, R.M., 2010. Planta Med. 76, 47.
Lee, S.M., Park, J.G., Lee, Y.H., Lee, C.G., Min, B.S., Kim, J.H., Lee, H.K., 2004. Biol. Pharm. Bull. 27, 1883.
Lee, S.S., Lin, B.F., Liu, K.C., 1996. Phytochemistry 43, 847.
Li, L.M., Liao, X., Peng, S.L., Ding, L.S., 2005. J. Integr. Plant Biol. 2005, 494.
Maciuk, A., Lavaud, C., Thépenier, P., Jacquier, M.J., Ghédira, K., Zéches-Hanrot, M., 2004. J. Nat. Prod. 67, 1639.
Matsuda, H., Murakami, T., Ikebata, A., Yamahara, J., Yoshikawa, M., 1999. Chem. Pharm. Bull. 47, 1744.
Meng, Y.J., Zhang, Y.W., Jiang, H.Y., Bao, Y.L., Wu, Y., Sun, L.G., Yu, C.L., Huang, Y.X., Li, Y.X., 2013. Biochem. Syst. Ecol. 50, 182.
Niu, X.F., Liu, X., Pan, L., Qi, L., 2011. Zhongguo Zhongyao Zazhi 36, 1319.
Pawlowska, A.M., Camangi, F., Bader, A., Braca, A., 2009. Food Chem. 112, 858.
Rosas, L.V., Cordeiro, M.S.C., Campos, F.R., Nascimento, S.K.R., Januário, A.H., França, S.C., Nomizo, A., Toldo, M.P.A., Albuquerque, S., Pereira, P.S., 2007. Braz. J. Med. Biol. Res. 40, 663.
Schühly, W., Heilmann, J., Calis, I., Sticher, O., 1999. Planta Med. 65, 740.
State Administration of Traditional Chinese Medicine, 1999. Zhonghua Bencao, vol. 5. Shanghai Science and Technology Publishing Company, Shanghai, China, p. 266.
Su, B.N., Cuendet, M., Farnsworth, N.R., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 2002. Planta Med. 68, 1125.
Suksamrarn, S., Suwannapoch, N., Aunchai, N., Kuno, M., Ratananuku, P., Haritakun, R., Jansakul, C., Ruchirawat, S., 2005. Tetrahedron 61, 1175.
Suksamrarn, S., Panseeta, P., Kunchanawatta, S., Distaporn, T., Ruktasing, S., Suksamrarn, A., 2006. Chem. Pharm. Bull. 54, 535.
Sun, Y.F., Liang, Z.S., Shan, C.J., Viernstein, H., Unger, F., 2011. Food Chem. 124, 1612.
Tian, S.S., Jiang, F.S., Zhang, K., Zhu, X.X., Jin, B., Lu, J.J., Ding, Z.S., 2014. Fitoterapia 92, 34.
Wang, J.R., Zhang, J., Yin, Z.Q., Liang, J.Y., Ye, W.C., 2008. Chin. J. Nat. Med. 6, 268.
Wang, M., Jiang, Y., Liu, H.L., Chen, X.Q., Wu, X., Zhang, D.Y., 2014. Nat. Prod. Res. 28, 70.
Wang, X.Q., Yang, X.J., Li, J.S., 2008. Zhongcaoyao 31, 853.
Weinges, K., Schick, H., 1995. Phytochemistry 38, 505.
Wu, G., Zhu, X.S., Yang, G.Z., Mei, Z.N., 2008. J. South-Center Univ. Natl. Nat. Sci. Ed. 27, 40.
Wu, T., Kong, D.Y., Li, H.T., 2004. Yaoxue Xuebao 39, 525.
Wu, Y., Zhang, J., Wang, D.Y., Liu, J.G., Hu, Y.L., 2013. Chem. Nat. Compd. 49, 677.

Xie, Y.Y., Xu, Z.L., Wang, H., Kano, Y., Yuan, D., 2011. J. Asian Nat. Prod. Res. 13, 1151.
Yagi, A., Okamura, N., Haraguchi, Y., Noda, K., Nishioka, I., 1978. Chem. Pharm. Bull. 26, 1798.
Yoshikawa, K., Shimono, N., Arihara, S., 1991. Tetrahedron Lett. 32, 7059.
Yoshikawa, M., Murakami, T., Ikebata, A., Wakao, S., Murakami, N., Matsuda, H., Yamahara, Y., 1997. Chem. Pharm. Bull. 45, 1186.